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Carcinogenesis studies of benzophenone in rats and mice

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Abstract

Benzophenone, an aryl ketone, is used primarily as a photoinitiator and fragrance enhancer. Groups of 50 male and 50 female F344 rats and B6C3 F1 mice were fed diets containing 0, 312, 625, and 1250 ppm benzophenone for 105 weeks. Survival of males exposed to 1250 ppm benzophenone was significantly less than that of controls. There was a positive trend in the incidence of renal tubule adenoma in male rats; these neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia. Increased incidences of mononuclear cell leukemia were observed in male rats exposed to 312 or 625 ppm benzophenone and in female rats exposed to 625 ppm benzophenone. Liver lesions observed included significantly increased incidences of hepatocytic centrilobular hypertrophy in all exposed groups of rats. In mice, survival of all exposed groups was generally similar to that of the control groups. In male mice, there were significantly increased incidences of hepatocellular adenoma in the 625 and 1250 ppm groups. In female mice, the incidences of hepatocellular adenoma in the 625 and 1250 ppm groups were higher than expected after adjusting for the lower body weights in these groups. The incidences of kidney nephropathy in exposed groups of female mice, as well as the severity of nephropathy in exposed groups of males, were significantly increased. The incidences of metaplasia of the olfactory epithelium were significantly increased in 1250 ppm mice. Rare histiocytic sarcomas were observed in female rats and mice in the 625 and 1250 ppm groups.

Under the conditions of these 2-year studies, there was some evidence of carcinogenic activity of benzophenone in male F344/N rats based on increased incidences of renal tubule adenoma. There was equivocal evidence of carcinogenic activity of benzophenone in female F344/N rats based on the marginal increased incidences of mononuclear cell leukemia and histiocytic sarcoma. There was some evidence of carcinogenic activity of benzophenone in male $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was some evidence of carcinogenic activity of benzophenone in female $B6C3F_1$ mice based on increased incidences of histiocytic sarcoma; the incidences of hepatocellular adenoma in female $B6C3F_1$ mice may have been related to benzophenone exposure.

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1. Introduction

Benzophenone, an aryl ketone, is used primarily as a photoinitiator and fragrance enhancer (NTP, 2000). It is also used in the manufacturing of insecticides, agricultural chemicals, and hypnotics, antihistamines, and other pharmaceuticals, as an ultraviolet curing agent in sunglasses and ink, as an additive in plastics, coatings, and adhesive formulations, and, as a flavor ingredient (NTP, 2000). Concentrations of benzophenone in food products range from 0.57 ppm in nonalcoholic beverages to 3.27 ppm in frozen dairy products; it may also be an ingredient in baked goods, soft candy, gelatins, and puddings.

Benzophenone was selected for toxicity and carcinogenicity testing based on the potential for occupational and

Abbreviation: NTP, National Toxicology Program.

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consumer exposure and a lack of chronic toxicity data. Incorporated in a sunscreen, benzophenone produced an allergic skin reaction in one patient, as assessed by photo patch testing (Cook and Freeman, 2001). Derivatives of benzophenone, particularly 2-hydroxy-4-methoxybenzophenone (benzophenone-3) and 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (benzophenone-4), are skin irritants that cause photoallergy and have been associated with allergic contact dermatitis (Alanko et al., 2001) and facial erythema (Nedorost, 2003).

The National Toxicology Program previously performed 14-week toxicity studies on benzophenone and published the results in a separate report (NTP, 2000). In the 14-week exposure to benzophenone at concentrations of 1250, 2500, 5000, 10,000 or 20,000 ppm in rats and mice, the liver and kidney were identified as the primary target organs of benzophenone toxicity in rats (NTP, 2000). In mice, the liver was the major target of toxicity. In rats, liver changes were observed at exposure concentrations greater than or equal to 5000 ppm, while in mice, microscopic changes in the liver were observed in all exposed groups.

The present studies were performed to characterize the chronic toxicity and carcinogenicity of benzophenone when administered in the diet to F344/N rats and B6C3F$_1$ mice.

2. Materials and methods

2.1. Chemical

Benzophenone (CAS# 119-61-9, ~99.5% pure) was obtained from Aldrich Chemical Company (Milwaukee, WI). The dose formulations were prepared at least once a month by mixing benzophenone with feed and were found to be stable for at least 35 days. Periodic analyses of the dose formulations were conducted using HPLC; all formulations were within 10% of the target concentrations. The formulations were stored at 5 °C for up to 35 days following preparation.

2.2. Experimental animals and housing conditions

All rodent studies were conducted at Battelle Memorial Institute, Columbus, OH. Animal use was in accordance with the United States Public Health Service policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985), and Battelle IACUC.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) and quarantined for 11–12 days (rats) or 25–26 days (mice) before administration of the chemical in environmentally controlled rooms (relative humidity of 50 ± 15%; temperature of 72 ± 3 °F; 12-h light/dark cycle). Feed and water were available *ad libitum*. The diet was irradiated NTP-2000 meal feed obtained from Zeigler Brothers (Gardners, PA). Rats were housed two or three (males) or five (females) per cage and mice were housed one (male) or five (females) per cage. Rats were approximately 6 weeks old and mice were approximately 8 weeks old on the first day of the studies.

2.3. Experimental design

Groups of 50 male and 50 female rats and mice were fed diets containing 0, 312, 625, and 1250-ppm benzophenone for 105 weeks. The highest dose was set at 1250 ppm based on the minimum toxicity observed at this level in the 14-week studies (NTP, 2000).

Animals were observed twice daily for moribundity and mortality and were weighed initially, on day 8, at 4-week intervals thereafter, and at the end of the study. Clinical findings were recorded on day 36, and at approximately 4-week intervals.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4–6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. To perform an extended evaluation of renal tubule proliferative lesions, additional sections of both kidneys in the residual formalin-fixed wet tissues from each male and female rat were embedded in separate paraffin blocks and step sectioned at 1 mm intervals. Up to eight step sections were examined for each animal. To identify staging of mononuclear cell leukemia in rats the following criteria were used. In stage 1, the spleen was not enlarged or was only slightly enlarged with small numbers of neoplastic mononuclear cells in the red pulp; none or very few mononuclear cells were observed in the liver sinusoids. No identifiable neoplastic cells were observed in other organs. In stage 2, the spleen was moderately enlarged with moderate to large numbers of neoplastic mononuclear cells in the red pulp; architectural features including lymphoid follicles and periarteriolar lymphocytic sheaths remained intact. There was minimal to moderate involvement of the liver. Neoplastic mononuclear cells may have been evident in blood vessels in other organs, but the aggregate/masses of neoplastic cells were generally limited to the spleen and liver. In stage 3, there was advanced disease with multiple organ involvement. The spleen was usually markedly enlarged with an effacement of normal architectural features by accumulated neoplastic mononuclear cells. Liver was moderately to markedly enlarged and nodular; hepatic parenchyma showed variable degenerative changes associated with the accumulation of neoplastic cells. There were accumulations of neoplastic cells in other organs including the lung, lymph nodes, kidney, brain, and adrenal gland.

2.4. Statistical methods

Organ and body weight data were analyzed with analysis of variance followed by the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). The Poly-*k*-test (Bailer and Portier, 1988; Piegorsch and Bailer, 1997; Portier and Bailer, 1989) was used to assess neoplasm and nonneoplastic lesion prevalence. Average severity values were analyzed for significance with the Mann–Whitney *U*-test (Hollander and Wolfe, 1973).

3. Results

3.1. Rats

Survival of 1250 ppm males was significantly less than that of the control group (4% compared to 44% in controls), while survival of exposed female groups was similar to that of the controls. Final body weights of high dose groups were 13–14% lower than controls for both males and females. Also, feed consumption was slightly lower both in high dose males and females. Dietary concentrations of 312, 625, and 1250 ppm resulted in average daily doses of approximately 15, 30, and 60 mg benzophenone/kg body weight to males and 15, 30, and 65 mg/kg to females. No clinical findings were attributed to benzophenone exposure.

Neoplastic and nonneoplastic histologic findings occurred in the kidney of males, and were interpreted to be due to benzophenone administration. Initially, single

sections of the left and right kidneys from each rat were examined microscopically. There was a positive trend in the incidence of renal tubule adenoma in males (Table 1). Renal tubule neoplasms were accompanied by significantly increased incidences of renal tubule hyperplasia in the 625 and 1250 ppm male dose groups (Table 1). Two females in the 625 ppm group and one in the 1250 ppm group had multiple renal tubule adenomas. Renal tubule hyperplasia was not significantly increased in dosed females. Renal tubule hyperplasia, adenoma, and carcinoma are thought to represent a continuum in the progression of proliferative lesions of the renal tubule epithelium. Because the incidences of renal tubule adenoma in exposed males and females and renal tubule hyperplasia in males were increased in the initial single kidney sections, additional kidney sections were examined to provide a more complete evaluation of the organ. After the extended evaluation (Table 1), a significant increase in the incidence of renal tubule adenoma was observed in 1250 ppm males, and increased incidences of hyperplasia were observed in all exposed groups of males. As a result of the extended evaluation, three renal tubule adenomas were observed in the control females; no additional neoplasms were observed in any group of females receiving benzophenone. The incidence of renal tubule hyperplasia in all groups of dosed females was significantly greater than that of the control group when the single and step section evaluations were combined (Table 1).

In males, the severity of chronic nephropathy increased with increasing benzophenone concentration, and the increases in all exposed groups were significant (Table 1). In exposed females, the severity of nephropathy was significantly increased in the 625 and 1250 ppm groups.

The increased severity of nephropathy in 1250 ppm males was associated with decreased survival after 80 weeks on study. Twenty-eight of 48 early deaths (58%) in this group, many moribund sacrificed, were attributed to nephropathy caused by benzophenone exposure. Because of the severe nephropathy, increases in several other findings usually associated with uremia, were observed at

Table 1
Incidences of neoplasms and nonneoplastic lesions of the kidney in F/344N rats exposed to benzophenone in feed for 2 years

	0 ppm	312 ppm	625 ppm	1250 ppm
Males				
Number examined	50	50	50	50
Single sections (standard evaluation)				
Renal tubule, hyperplasia[a]	1 (1.0)[b]	5 (1.4)	20** (1.5)	23** (1.3)
Nephropathy	50 (1.3)	45 (2.4)▲	50 (3.3) ▲	50 (3.8) ▲
Renal tubule, adenoma	1	1	2	4
Renal tubule, carcinoma	0	1	0	0
Renal tubule, cyst	0	0	1	9**
Step sections				
Renal tubule hyperplasia	2 (1.0)	8* (1.1)	26** (2.0)	37** (2.2)
Renal tubule, adenoma	1	1	5	4
Renal tubule, carcinoma	0	1	0	0
Single and step sections (combined)				
Renal tubule, hyperplasia	3 (1.0)	11* (1.3)	30** (1.8)	40** (2.1) ▲
Renal tubule, adenoma	2	2	7	8*
Renal tubule, carcinoma	0	1	0	0
Females				
Number examined	50	50	50	50
Single sections (standard evaluation)				
Renal tubule, hyperplasia	0	1 (4.0)	1 (3.0)	1 (4.0)
Nephropathy	47 (1.1)	49 (1.4)	48 (1.7) ▲	49 (2.0) ▲
Renal tubule, adenoma, multiple	0	0	2	1
Step sections				
Renal tubule hyperplasia	1 (1.0)	7* (1.1)	9* (2.1)	6 (1.7)
Renal tubule, adenoma	3	0	2	1
Single and step sections (combined)				
Renal tubule, hyperplasia	1 (1.0)	8* (1.5)	10** (2.2)	7* (2.0)
Renal tubule, adenoma, multiple	0	0	1	1
Renal tubule, adenoma (includes multiple)	3	0	2	1

* Significantly different ($p \leqslant 0.05$) from the control group by the Poly-3 test.
** $p \leqslant 0.01$.
▲ Severity significantly different ($p \leqslant 0.05$) from the control group by the Mann–Whitney U-test.
[a] Number of animals with lesions.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.

multiple sites in male rats. These secondary findings included increased mineralization of blood vessels and basement membranes including kidney cortex, heart, seminal vesicles, forestomach, glandular stomach, and lung, in addition to parathyroid gland hyperplasia and fibrous osteodystrophy in bone (data not shown).

Increased incidences of mononuclear cell leukemia occurred in exposed groups of females, and the increase was significant at 625 ppm (Table 2). Male rats exposed to 312 or 625 ppm also had significantly increased incidences of mononuclear cell leukemia, although that of 1250 ppm males was slightly decreased (Table 2). The incidences in all exposed groups of females and 312 and 625 ppm males exceeded the range reported for historical controls from feed studies (historical incidence: 112/460, range 12–38%). Mononuclear cell leukemia was graded according to the extent of involvement of the spleen, liver, lung, and other organs. Similar criteria have been used for previous NTP studies (NTP, 1986). The involvement of spleen, liver, and other organs in female rats increased with increased levels of benzophenone exposure. The extent of multiple organ involvement of leukemia in male rats decreased in the higher exposure groups.

A few histiocytic sarcomas occurred in the 625 and 1250 ppm groups of females (Table 2). This neoplasm has not been observed in historical feed study controls given NTP-2000 diet, and has been observed in only one out of 1209 historical controls for all exposure routes in NTP studies. Histiocytic sarcomas were observed in the lung and livers of all three affected rats. Cells in these neoplasms exhibited variations in size and shape and had high cytoplasmic-to-nuclear ratios. Another characteristic histologic feature observed in neoplasms in two rats was necrotic areas surrounded by rows of neoplastic cells. Prominent multinucleated giant cells were also present in the tumors in two animals. Histologic features differed from animal to animal and from site to site in the same animal. Neoplastic histiocytic cells infiltrated the liver, diffusely expanding the hepatic parenchyma (a). In the lung, intravascular masses and perivascular infiltrates of neoplastic histiocytic cells were observed in all affected rats.

Nonneoplastic lesions occurred in the liver and were considered to be treatment-related (Table 3). The incidences of liver centrilobular hepatocellular hypertrophy in all exposed groups of males and females were significantly greater than those in the control groups. This hepatocellular hypertrophy is consistent with the induction of P450 enzymes previously observed in the 14-week study (NTP, 2000). Incidences of cystic degeneration of hepatocytes and chronic active inflammation in 625 and 1250 ppm males, and bile duct hyperplasia in all exposed groups of females were significantly greater than those in the control groups. The incidences of chronic active inflammation in all exposed female groups were significantly decreased.

Statistically significant decreases in the incidences of mammary gland fibroadenoma (including multiple) occurred in females exposed to 625 or 1250 ppm benzophenone (0 ppm, 27/50; 312 ppm, 24/50; 625 ppm, 15/50; 1250 ppm, 7/50). Multiple fibroadenomas were significantly decreased in the 1250 ppm group (6/50; 4/50; 3/50; 0/50). The incidence of fibroadenoma (including multiple) combined in the 1250 ppm group is lower than expected after adjusting for decreased body weight (14.7 expected, 7 observed) and is less than the historical control range from feed studies and from all routes combined (feed:

Table 2
Incidences and stages of mononuclear cell leukemia and incidences of histiocytic sarcoma in F/344N rats exposed to benzophenone in feed for 2 years

	0 ppm	312 ppm	625 ppm	1250 ppm
Males				
Mononuclear cell leukemia	27/50[a]	41/50**	39/50**	24/50
# of animals surviving at end of study	22	27	31	2
# with grade 1 mononuclear cell leukemia	1	8	7	11
# with grade 2 mononuclear cell leukemia	4	8	18	2
# with grade 3 mononuclear cell leukemia	22	25	14	11
Total with mononuclear cell leukemia	27	41	39	24
Average staging grade	2.8	2.4*	2.2**	2.0**
Females				
Mononuclear cell leukemia	19/50	25/50	30/50*	29/50
# of animals surviving at end of study	32	38	37	34
# with grade 1 mononuclear cell leukemia	10	13	11	10
# with grade 2 mononuclear cell leukemia	6	4	7	8
# with grade 3 mononuclear cell leukemia	3	8	12	11
Total with mononuclear cell leukemia	19	25	30	29
Average staging grade	1.6	1.8	2.0	2.0
Histiocytic sarcoma	0/50	0/50	1/50	2/50

* Significantly different ($p \leqslant 0.05$) from the control group by the Poly-3 test.
** $p \leqslant 0.01$.
[a] Number of animals with neoplasm per number of animals necropsied.

Table 3
Incidences of nonneoplastic lesions of the liver in F/344N rats exposed to benzophenone in feed for 2 years

	0 ppm	312 ppm	625 ppm	1250 ppm
Males				
Number examined	50	50	50	50
Hepatocyte, centrilobular hypertrophy[a]	0	17** (1.3)[b]	31** (1.8)	19** (1.5)
Degeneration, cystic	8 (1.3)	11 (1.0)	20** (1.3)	15* (1.2)
Inflammation, chronic active	22 (1.9)	21 (1.6)	35** (1.9)	33* (1.8)
Females				
Number examined	50	50	50	50
Hepatocyte, centrilobular hypertrophy	0	27** (1.0)	30** (1.3)	33** (2.0)
Bile duct, hyperplasia	10 (1.3)	35** (1.2)	39** (1.4)	40** (1.6)
Inflammation, chronic active	46 (1.5)	38* (1.5)	29** (1.3)	30** (1.4)

* Significantly different ($p \leqslant 0.05$) from the control group by the Poly-3 test.
** $p \leqslant 0.01$.
[a] Number of animals with lesions.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.

213/460 (44.3% ± 11.9%), range 28–55%; all routes: 567/1209 (45.9% ± 12.0%), range 28–72%).

3.2. Mice

The administration of benzphenone did not significantly affect survival or food consumption. Final body weights were similar to those of the controls throughout the study except in high dose females (14% less than controls). Dietary concentrations of 312, 625, and 1250 ppm benzophenone resulted in average daily doses of 40, 80, and 160 mg benzophenone/kg body weight to males and 35, 70, and 150 mg/kg to females. No clinical findings were attributed to benzophenone exposure.

There was a positive trend in the incidences of hepatocellular adenoma in male mice; the incidence in the 625 and 1250 ppm groups was significantly greater than that in the controls (Table 4). Statistically significant increases in the incidences of multiple hepatocellular adenomas occurred in all exposed male mice. However, the incidence of carcinomas was not increased. Hepatoblastomas were also observed in exposed males. The incidence of hepatocellular adenoma in 625 and 1250 ppm female mice was increased, but differences were not significant relative to

Table 4
Incidences of nonneoplastic and neoplastic lesions of the liver in B6C3F$_1$ mice exposed to benzophenone in feed for 2 years

	0 ppm	312 ppm	625 ppm	1250 ppm
Males				
Number examined	50	50	50	50
Hepatocellular adenoma, multiple[a]	2	8*	8*	12**
Hepatocellular adenoma (includes multiple)	11	15	23*	23*
Hepatocellular carcinoma	8	5	6	6
Hepatoblastoma	0	1	1	3
Clear cell focus	2	7	7	12**
Eosinophilic focus	5	8	11	10
Mixed cell focus	8	9	15	13
Hepatocyte, centrilobular, hypertrophy	0	44** (2.0)[b]	50** (2.0)	48** (3.0)
Hepatocyte, multinucleated	0	41** (1.4)	47** (1.5)	48** (1.8)
Hepatocyte, necrosis	1 (1.0)	6 (1.7)	8* (1.8)	8* (1.3)
Inflammation, chronic active	33 (1.0)	47** (1.1)	44** (1.2)	42* (1.1)
Hepatocyte, degeneration, cystic	0	0	5* (1.2)	30** (1.9)
Females				
Number examined	50	50	50	50
Hepatocellular adenoma, multiple	1	1	3	3
Hepatocellular adenoma (includes multiple)	5	4	10	8
Hepatocellular carcinoma	0	1	0	1
Eosinophilic focus	2	2	7	7
Hepatocyte, centrilobular, hypertrophy	0	29** (2.0)	44** (2.0)	37** (2.9)

* Significantly different ($p \leqslant 0.05$) from the control group by the Poly-3 test.
** $p \leqslant 0.01$.
[a] Number of animals with lesions.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.

controls. The incidence of liver tumors in control mice in NTP studies, primarily hepatocellular adenomas, has been found to be positively associated with body weight (Haseman et al., 1997). When adjusted for the decreased body weight gains of exposed female mice, there were more hepatocellular adenomas in the 625 and 1250 ppm groups than expected (0 ppm: 6.8 expected, 5 observed; 312 ppm: 7.0 expected, 4 observed; 625 ppm: 6.2 expected, 10 observed; 1250 ppm: 4.3 expected, 8 observed). Exposed males and females had increased incidences of eosinophilic foci, and males had increases in clear and mixed cell foci. However, only the increase in clear cell foci in 1250 ppm males was significantly different from controls. Statistically significant increases in centrilobular hepatocyte hypertrophy were observed in all exposed groups of mice (Table 4). The livers of most exposed males had multinucleated hepatocytes and increases in necrosis and chronic active inflammation. The 625 and 1250 ppm male groups had significant increases in the incidences of cystic degeneration of hepatocytes. This lesion is reported with a low incidence as spontaneous finding in aged mice.

In females, there was a positive trend in the incidence of histiocytic sarcoma and the incidence in the 625 ppm group was significantly greater than that in the controls (Table 5). Only two histiocytic sarcomas have been observed in NTP historical feed study controls, and the incidence in the 625-ppm group exceeded the historical control range for all routes (Table 5). In the current 2-year study, only females were affected, and the liver and lung were involved in all affected females. The histiocytic sarcomas were highly invasive in all three 1250 ppm mice. Multiple organs had neoplastic histiocytic lesions. Ovary, uterus, spleen, adrenal gland, kidney, urinary bladder, and multiple lymph nodes were affected in all three animals. Variation in the size and shape of some neoplastic cells and high cytoplasmic-to-nuclear ratios were observed. Exposed female mice had significantly increased incidences of kidney nephropathy accompanied by mineralization (Table 5). The severity of nephropathy was significantly increased in all exposed groups of male mice, and the nephropathy was accompanied by significantly increased incidences of cortex cysts in the 625 and 1250 ppm groups. Tubular degeneration, tubular regeneration, interstitial inflammation, dilatation of renal tubules, intratubular protein casts, and subcapsular regions of interstitial fibrosis scars characterized nephropathy.

Table 5
Incidences of selected nonneoplastic and neoplastic lesions in B6C3F$_1$ mice exposed to benzophenone in feed for 2 years

	0 ppm	312 ppm	625 ppm	1250 ppm
Males				
Number examined	50	50	50	50
Kidney				
Nephropathy[a]	49 (1.2)[b]	48 (1.4)▲	50 (1.7)▲	50 (3.0) ▲
Cortex, cyst	4	8	12*	22**
Nose				
Olfactory epithelium, metaplasia	0	2 (1.0)	2 (1.0)	24** (1.2)
Spleen				
Lymphoid follicle hyperplasia, lymphoid	17 (2.1)	31** (2.5)	34** (2.0)	32** (2.2)
Testes				
Mineralization	0	1 (1.0)	4 (1.0)	12** (1.1)
Females				
Number examined	50	50	50	50
Histiocytic sarcoma[c]	0	0	5*	3
Kidney				
Nephropathy	21 (1.2)	33** (1.1)	31* (1.5)	30* (1.7) ▲
Mineralization	15 (1.0)	31** (1.0)	36** (1.1)	49** (1.5)
Nose				
Olfactory epithelium, metaplasia	0	0	0	39** (1.7)
Spleen				
Hematopoietic cell proliferation	16 (2.6)	35** (2.1)	32** (2.4)	27* (2.8)
Lymphoid follicle, hyperplasia, lymphoid	24 (2.5)	36** (2.5)	37** (2.7)	22 (2.9)

* Significantly different ($p \leqslant 0.05$) from the control group by the Poly-3 test.
** $p \leqslant 0.01$.
▲ Significantly different severity ($p \leqslant 0.05$) from the control group by the Mann–Whitney U-test.
[a] Number of animals with lesions.
[b] Average severity grade of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.
[c] Historical incidence for 2-year studies with feed controls, given NTP-2000 diet (mean ± standard deviation): 2/459 (0.3% ± 0.8%), range 0–2%; all routes 18/1258 (1.5% ± 2.2%), range 0–8%.

The incidences of metaplasia of the olfactory epithelium were significantly increased in 1250-ppm male and female groups (Table 5). The metaplasia was characterized by a replacement of normal olfactory epithelium by a single layer of ciliated columnar epithelium resembling normal respiratory epithelium.

The incidence of hematopoietic cell proliferation in all exposed groups of female mice was significantly greater than that of the controls (Table 5). Hyperplasia of lymphoid follicles was significantly increased in all exposed groups of males and in 312 and 625 ppm females (Table 5).

A significantly increased incidence of testes mineralization was present in 1250 ppm males (Table 5). The mineralization was not associated with degeneration of the germinal epithelium.

4. Discussion

In the current 2-year benzophenone studies, the target organs of toxicity were liver, kidney, nose, and testes. Neoplastic responses occurred in the kidney, liver, and hematopoietic system.

Rats exposed to benzophenone exhibited a positive trend in the incidences of renal tubule adenoma. The NTP has shown that examination of the entire kidney, by step sectioning of residual tissues, enables a more precise evaluation of the potential chemical-related induction of renal proliferative lesions than observations made from single sections, particularly when the proliferative lesions are small and identified only by microscopic examination (Eustis et al., 1994). For benzophenone, this extended evaluation of the male rat kidney showed significant increases in the incidences of renal tubule adenomas and hyperplasia in exposed groups. Incidences of renal tubule hyperplasia in all exposed female groups were significantly greater than that of the control group when the single and step section evaluations were combined. Renal tubule hyperplasia, adenoma, and carcinoma are considered to comprise a biological and morphological continuum in the progression of proliferative changes of the renal tubule epithelium. Both renal tubule hyperplasia and adenomas are characterized by increased numbers of relatively normal appearing tubular epithelial cells. Adenomas are large discrete lesions that are greater than five tubule diameters to 1 mm or more in size. Adenomas tend to have complex cellular patterns. Carcinomas are usually larger than adenomas with irregular borders, a prominent blood supply, and cellular anaplasia.

The pathogenesis of chemically induced renal tubule neoplasms has not been determined; however it appears to be complex with genotoxic and nongenotoxic modes (Barrett and Huff, 1991; Hard, 1998; Short, 1993). Data from retrospective reviews of NTP 2-year carcinogenesis studies suggest that an increased severity of nephropathy may contribute to overall tumor response (Seely et al., 2002). However, any contribution appears to be marginal, and additional factors are likely involved.

In female rats, the incidence of mononuclear cell leukemia was significantly increased in the 625 ppm group. Male rats exposed to 312 or 625 ppm benzophenone also exhibited significantly increased incidences of mononuclear cell leukemia. There was not a statistically significant increase in 1250 ppm males; however, this is a late developing neoplasm and only two of the 50 animals in this group survived to the end of the study. It is possible that the early deaths of these animals precluded the ability to determine if benzophenone exposure increased mononuclear cell leukemia in male rats in a dose-dependent manner. Mononuclear cell leukemia is a common neoplasm in F344/N rats and has occurred at incidences of 30–68% in male controls from 2-year NTP feed studies and 12–38% in control females. Because of this variability, staging of the neoplasms is often done as a way of further evaluating a chemical-related increase in incidence. In this case, staging of the lesions provided no support to indicate that benzophenone was enhancing the progression of these lesions; therefore, the increased incidences were considered equivocal evidence of carcinogenicity.

Benzophenone exposure resulted in a positive trend in the incidence of histiocytic sarcoma in female mice, and one 625 ppm and two 1250 ppm female rats had histiocytic sarcomas. This neoplasm is extremely rare; none have been observed in historical feed study control rats and only two have been observed in feed study control mice given the NTP-2000 diet. Histiocytic sarcomas are classified as hematopoietic tumors of the mononuclear phagocyte system based upon the morphology of the neoplastic cells and the presence of lysozyme, Mac-2, and mononuclear phagocyte antigens. They are slightly more common in female than male mice and in mice than rats (Frith et al., 1993). The histiocytic sarcomas observed in mice exposed to benzophenone involved the liver and lung. In the 1250 ppm female mice, the histiocytic sarcomas were highly invasive. Multiple organs throughout the body had neoplastic histiocytic lesions. All affected rats exposed to benzophenone had lung lesions. Only one rat in the 625 ppm group had organs affected throughout the body.

Chemical-associated increases in the incidences of histiocytic sarcomas are rare in NTP studies; however increased incidences have been observed in the studies with 1,3-butadiene (NTP, 1993), tetrafluoroethylene (NTP, 1996a), and phenolphthalein (NTP, 1996b). The rarity of histiocytic sarcomas, combined with the fact that mice displayed a positive trend in the incidence of this lesion suggest that the occurrences in female rats may be related to benzophenone exposure, but the low number of neoplasms observed makes this an uncertain finding.

Male and female mice in all exposed groups had increased incidences of spleen hematopoietic cell proliferation. The incidence in the control female group in this study is consistent with previous NTP studies (Ward et al., 1999). Increased hematopoietic cell proliferation has been associated with anemia and chronic inflammatory lesions. Evidence of an anemia with minimal severity was

observed in rats and mice during the 14-week studies at higher doses than were used in the 2-year study (NTP, 2000).

Increases in the incidences of hepatocellular adenoma were observed in male and female mice. Hepatoblastomas were also observed in exposed males; however the increased incidence was not statistically significant. Hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas represent a biological and morphological continuum in progression of proliferative lesions. Because the malignant potential of hepatoblastomas and hepatocellular carcinomas appears similar and hepatoblastomas are often observed within hepatocellular neoplasms (mostly carcinomas), it is appropriate to combine the incidences of hepatoblastoma with those of adenoma and carcinoma when interpreting the carcinogenic potential of a chemical. The combined incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma was significantly increased in 1250 ppm males, and the incidences showed a positive trend.

In the current study, the incidences of metaplasia of the olfactory epithelium were significantly increased in the high dose male and female mice. This was a species-specific effect, as rats did not display similar lesions, possibly because of differences in the anatomy of the rat nasal cavity and potential lower relative exposures to benzophenone in rats. The mechanism by which benzophenone caused this lesion is unknown; however, enzymatic metabolism of benzophenone in the olfactory epithelium, which has a high activity of cytochrome P450, may be involved. Some compounds, such as phosphodiesterase inhibitors, that require metabolic activation by the cytochrome P450 enzyme system have been shown to cause olfactory epithelial injury, chronic hyperplastic/regenerative lesions, and olfactory neoplasms following oral or inhalation exposure in rodents (Pino et al., 1999).

Decreases in the incidences and multiplicities of mammary gland fibroadenoma were observed in female rats exposed to benzophenone. Fibroadenomas are the most common spontaneous neoplasm of the mammary gland in female rats, occurring in 213/460 (46%, range 28–55%) NTP feed study control animals. The incidence of mammary gland tumors in NTP studies has been found to be positively associated with body weight. However, the decreased incidence of mammary gland tumors in this study could not be attributed to decreased body weights of exposed females, as the 1250 ppm females had significantly lower incidences of this neoplasm after correcting for decreased body weight (Haseman et al., 1997). Interestingly, benzophenone-based derivatives have shown impressive inhibitory activity of steroid sulfatase, an enzyme that regulates the formation of estrone and subsequent conversion to estradiol, and may be developed for therapeutic use in the treatment of hormone-dependent breast cancer (Hejaz et al., 2004).

In conclusion, there was some evidence of carcinogenic activity of benzophenone in male F344/N rats based on increased incidences of renal tubule adenoma. There was equivocal evidence of carcinogenic activity of benzophenone in female F344/N rats based on the marginal increased incidences of mononuclear cell leukemia and histiocytic sarcoma. There was some evidence of carcinogenic activity of benzophenone in male $B6C3F_1$ mice based on increased incidences of hepatocellular neoplasms, primarily adenoma. There was some evidence of carcinogenic activity of benzophenone in female $B6C3F_1$ mice based on increased incidences of histiocytic sarcoma; the incidences of hepatocellular adenoma in female $B6C3F_1$ mice may have been related to benzophenone exposure.

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